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Abstract

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KEYWORDS: host range, helper leukemia virus, pseudotype MSV

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RESTRICTION OF HOST RANGE OF XENOTROPIC PSEUDOTYPE MURINE SARCOMA VIRUS BY HELPER LEUKEMIA VIRUS

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Abstract. We investigated the restriction of the host range to infectivity of MSV by helper leukemia virus *in vivo*. When newborn SD-rats were inoculated intracerebrally, subcutaneously, intraperitoneally or intramuscularly with xenotropic pseudotype Kirsten MSV, Ki-MSV(BV2), either brain tumors or myogenic sarcomas were induced, depending upon the route of inoculation. However, no tumors developed in SW-Icr mice inoculated with Ki-MSV(BV2) either intracerebrally or intramuscularly at birth. Ecotropic Ki-MSV(Ki-MuLV) induced myogenic sarcomas in mice when inoculated intramuscularly and also induced brain tumors and myogenic sarcomas in rats when inoculated intracerebrally and intramuscularly, respectively. Thus, the host range of pseudotype MSV appeared to depend on a helper leukemia virus.

Key words : host range, helper leukemia virus, pseudotype MSV, sarcoma virus

Brain tumors developed frequently in Sprague-Dawley (SD) rats inoculated intracerebrally (i.c.) with xenotropic pseudotype MSV, Ki-MSV(BV2), pre- or neonatally. Brain tumors thus induced included glioblastoma multiforme and hemangioendotheliomas. Cerebellar tumors showed hemangiomas associated with multinucleated giant cells in most cases (1). When rats were inoculated intramuscularly (i.m.), subcutaneously (s. c.) or intraperitoneally (i. p.), brain tumors were also noticed. Ki-MSV(BV2) induced brain tumors and sarcomas in rats but not in mice. However, ecotropic pseudotype MSV, Ki-MSV(Ki-MuLV) prepared *in vitro* by rescuing Ki-MSV with ecotropic Ki-MuLV, induced brain tumors or myogenic sarcomas in both rats and mice when inoculated i.c. and i.m., respectively (2, 3). Thus, the host range of pseudotype MSV appeared to depend on a helper virus (3, 4).

MATERIALS AND METHODS

Animals. Sprague-Dawley (SD) rats and Swiss Icr or C3H mice were obtained from CLEA

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Viruses. NRK cells producing xenotropic pseudotype Ki-MSV(BV2) (5) were obtained from Dr. Aaronson and Dr. Hino (NIH, Bethesda, Md, USA). Ki-NRK (nonproducer) cells and Ki-MuLV/NRK cells were presented by Dr. Aaronson.

Rescuing of MSV with xenotropic or ecotropic helper virus. Defective MSV were rescued with ecotropic or xenotropic MuLV, using nonproducing Ki-NRK cells (6) isolated from transformed NRK cells with Ki-MSV.

Human A673 cells (7) producing xenotropic MuLV, (BV2), and non-producer Ki-NRK cells were cultured together at a cell concentration of 10^5/ml each to prepare Ki-MSV(BV2). Similarly, NRK cells producing ecotropic MuLV, (Ki-MuLV), and Ki-NRK (non-producer) cells were co-cultured at the same concentrations to obtain Ki-MSV(Ki-MuLV). Focus assay (4) was done with filtrates of the supernatants of the co-cultured cells. A newly obtained xenotropic pseudotype MSV, Ki-MSV(BV2) transformed both mink cells (8) and NRK cells (9) with a titer of $10^{2.3}$ FFU/ml on mink cells and $10^{3.1}$ FFU/ml on NRK cells. This xenotropic pseudotype MSV transformed neither NIH/3T3 nor BALB/3T3 cells (10). In contrast to this virus, ecotropic pseudotype MSV, Ki-MSV(Ki-MuLV) transformed BALB/3T3 and NRK cells but did not transform mink cells. The titration of ecotropic pseudotype MSV thus prepared was $10^{3.7}$ FFU/ml on BALB/3T3 cells and $10^{4.5}$ FFU/ml on NRK cells.

Tissue culture. NRK cells, BALB/3T3 cells, NIH/3T3 cells, Ki-NRK cells (non-producer), A673 cells (human cells) were cultured in Eagle's medium with 10 percent fetal calf serum. The titration of xenotropic pseudotype MSV and xenotropic MuLV was conducted on mink cells (11) and mink S+L- cells (12), respectively, using RPMI 1640 medium with 10 percent fetal calf serum.

Inoculation of the viruses into animals. Ki-MSV(BV2) or Ki-MSV(Ki-MuLV) was inoculated intracerebrally (0.05 ml), intraperitoneally (0.2 ml) or intramuscularly (0.2 ml) into newborn SD-rats, and intracerebrally (0.02 ml) or intramuscularly (0.1 ml) into newborn SW Icr and C3H mice.

Transplantation. Sarcomas induced in the thighs of SW Icr mice with Ki-MSV(Ki-MuLV) were transplanted into the same strain of mice.

Autopsy. Organs were fixed in 10 % buffered formalin solution after autopsy, and embedded in paraffin. The sections were stained with Hematoxylin-Eosin, Mallory PTAH, PAS, silver stain (Gomori's modified method) and Aleucian blue.

RESULTS

Susceptibility of SD-Rats to Xenotropic Pseudotype Ki-MSV(BV2)

Ki-MSV(BV2) was inoculated intracerebrally, subcutaneously, intraperitoneally, and intramuscularly into newborn SD-rats. As shown in Table 1, xenotropic pseudotype Ki-MSV, Ki-MSV(BV2), induced sarcomas and brain tumors in SD-rats. Details of the results were given in a previous paper (13).

Susceptibility of SW Icr Mice to Ki-MSV(BV2)

Ki-MSV(BV2) was inoculated intracerebrally and intramuscularly into newborn SW Icr mice. Thirty newborn SW Icr mice were inoculated with Ki-MSV (BV2) intracerebrally (0.02 ml) and twenty-four SW Icr mice intramuscularly in the thigh (0.1 ml). All the animals survived and were healthy for over one year. Thus, the mice were not susceptible to xenotropic pseudotype Ki-MSV rescued

TABLE 1. INOCULATION OF KI-MSV(BV2) INTO NEWBORN OR FETAL SD-RATS BY VARIOUS ROUTES

Route of inoculation	Age of rats	Number of animals	Cerebral tumor (%)	Cerebellar tumor (%)	Enlargement of spleen(%)	Enlargement of liver(%)	Hemorrhage in skin (%)	ALP (Day)
IC	Newborn	38	37 (97.4)	17 (44.7)	7 (18.4)	2 (5.3)	0 (0)	37
	Fetus	34	26 (76.5)	31 (91.2)	15 (44.1)	16 (47.1)	1 (3.0)	24
SC	Newborn	21	10 (47.6)	10 (47.6)	8 (38.1)	9 (42.9)	9 (42.9)	22
	Fetus	39	27 (69.2)	30 (76.9)	34 (87.2)	29 (74.4)	20 (51.3)	24
IP	Newborn	19	4 (21.1)	12 (63.2)	3 (15.8)	0 (0.)	5 (26.3)	15
	Fetus	14	4 (28.6)	3 (21.4)	3 (21.4)	1 (7.1)	1 (7.1)	34
IM*	Newborn	17	1 (5.9)	1 (5.9)	10 (58.8)	4 (23.5)	0 (0)	76

* Ki-MSV (BV2) induced sarcomas at the site of inoculation in 88.2% (15/17) of the animals.
ALP : Avarage latent period.

TABLE 2. INOCULATION OF KI-MSV(BV2) INTO SW-ICR MICE BY VARIOUS ROUTES

Route of inoculation	Number of animals inoculated	Thigh tumor	Brain tumor	ALP (Day)
IC	7	0	0	*
〃	13	0	0	
〃	10	0	0	
Total	30	0	0	
IM	8	0	0	*
〃	7	0	0	
〃	9	0	0	
Total	24	0	0	

* All animals survived and were healthy over one year.
ALP : See Table 1.

with helper virus, BV2 (Table 2).

Susceptibility of Mice to Ecotropic Pseudotype Ki-MSV(Ki-MuLV).

Intramuscular inoculation of Ki-MSV(Ki-MuLV) into newborn mice. As shown in Table 3, when the mice were inoculated with Ki-MSV(Ki-MuLV) intramuscularly in the thigh, sarcomas developed at the site of inoculation in 100 percent (7/7) of Swiss Icr mice and 84 percent (16/19) of C3H mice, after average latent

TABLE 3. INTRAMUSCULAR INOCULATION OF KI-MSV(KI-MULV) INTO NEWBORN MICE

Strain of mice	Number of animals inoculated	Thigh tumor	Incidence %	ALP (Day)
SW	7	7	100	19
C3H	6	6	100	16
〃	5	2	40	26
〃	8	8	100	18
Total(C3H)	19	16	84[a]	18[a]

ALP : See Table 1. *a* These numbers were calculated by averaging the total numbers of C3H mice.

TABLE 4. INTRAMUSCULAR INJECTION OF A CELL SUSPENSION OF KI-MSV(KI-MULV) INDUCED MOUSE SARCOMAS

Number of animals injected	"Take" (%)	ALP (Day)
16	13 (81)	20

ALP : See Table 1.

TABLE 5. INTRACEREBRAL INOCULATION OF KI-MSV(KI-MULV) INTO NEWBORN SD-RATS

Number of animals	Cerebral tumor (%)	Cerebellar tumor (%)	Enlargement of spleen(%)	Enlargement of liver (%)	Hemorrhage in lungs(%)	ALP (Day)
10	10	5	0	0	3	21
8	8	8	5	1	2	24
7	7	7	5	1	3	21
13	13	8	12	4	4	26
10	10	10	6	4	3	21
7	7	7	6	5	4	32
9	9	8	6	6	2	22
64	64 (100)	53 (82.8)	40 (62.5)	21 (32.8)	21 (32.8)	24

ALP : See Table 1.

periods of 19 and 18 days, respectively.

Transplantation of Ki-MSV(Ki-MuLV) induced mouse sarcoma into mice. When sarcomas induced by Ki-MSV(Ki-MuLV) in Swiss Icr mice were transplanted into the thighs of newborns of the same strain of mice, 81 % (13/16) of them took at the site of transplantation, as shown in Table 4.

Susceptibility of SD-Rats to Ecotropic pseudotype Ki-MSV(Ki-MuLV).

When Ki-MSV(Ki-MuLV) was inoculated intracerebrally in newborn SD-

TABLE 6. INTRAPERITONEAL INOCULATION OF KI-MSV(KI-MULV) INTO NEWBORN SD-RATS

Number of animals	Cerebral tumor (%)	Cerebellar tumor (%)	Enlargement of spleen (%)	Enlargement of liver (%)	Hemorrhage in lungs (%)	Thoracic fluids (%)	ALP (Day)
7	2	4	4	6	7	7	16
8	8	8	8	8	8	5	25
6	5	4	5	5	6	5	16
21	15 (71.4)	16 (76.2)	17 (81.0)	19 (90.5)	21 (100)	17 (81.0)	19

ALP : See Table 1.

TABLE 7. INTRAMUSCULAR INOCULATION OF KI-MSV(KI-MULV) INTO NEWBORN SD-RATS

Number of animals	Thigh tumor (%)	Cerebral tumor (%)	Cerebellar tumor (%)	Enlargement of spleen (%)	Enlargement of liver (%)	Hemorrhage in lungs (%)	Enlargement of lymph-nodes (%)	ALP (Day)
2	2	2	2	2	2	2	1	21
13	12	11	13	11	8	11	8	32
2	2	2	2	2	1	1	1	31
5	5	5	5	5	3	4	4	33
7	5	5	3	5	2	4	2	30
7	7	6	4	7	4	6	4	34
7	7	6	6	7	4	7	7	42
5	5	3	4	5	3	5	3	46
48	45 (93.8)	40 (83.3)	39 (81.3)	44 (91.7)	27 (56.3)	38 (79.2)	30 (62.5)	34

ALP : See Table 1.

rats, cerebral tumors developed in 100 percent (64/64) and cerebellar tumors in 82.8 percent (53/64), after an average of 24 days, as shown in Table 5. Moderately enlarged spleens and livers were also noted.

Intraperitoneal inoculation of Ki-MSV(Ki-MuLV) into newborn SD-rats. As shown in Table 6, tumors were found in the cerebrum in 71.4 percent (15/21) and in the cerebellum in 76.2 percent (16/21) of SD-rats inoculated with Ki-MSV(Ki-MuLV) intraperitoneally at birth. In addition enlargement of the spleen was observed in 81 percent (17/21), enlargement of the liver in 90.5 percent (19/21), hemorrhages in the lungs in 100 percent (21/21) and hemorrhagic pleural fluids in thoracic cavity in 81 percent (17/21), after an average of 19 days.

Intramuscular inoculation of Ki-MSV(Ki-MuLV) into newborn SD-rats. As shown in Table 7, sarcomas developed at the site of inoculation in 93.8 percent (45/48) of rats inoculated with Ki-MSV(Ki-MuLV) in the thigh at birth. Brain tumors

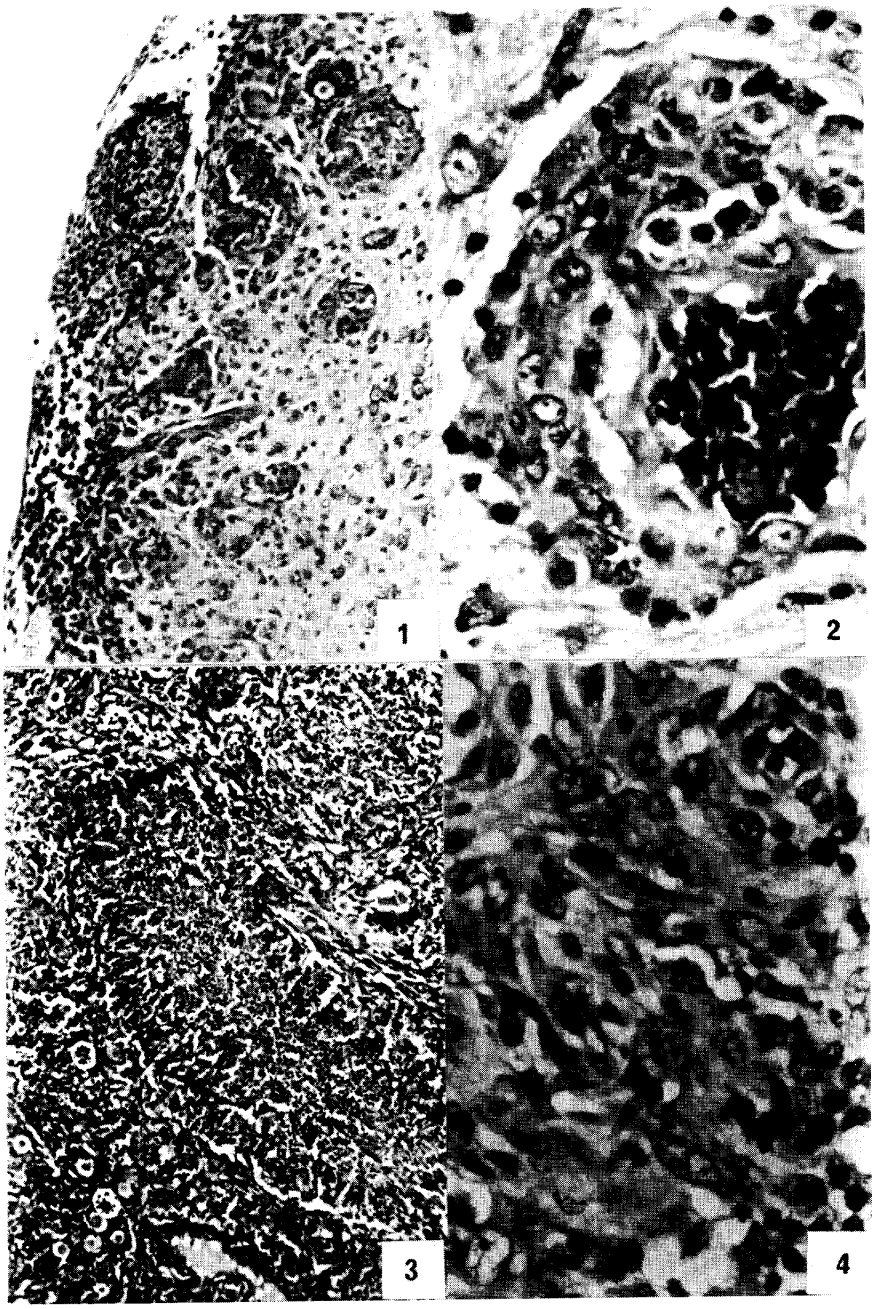

Fig. 1-4. Microscopic observations of Ki-MSV (Ki-MuLV) induced rat brain tumors.

also were noted in the cerebrum in 83.3 percent (40/48) and the in cerebellum in 81.3 percent (39/48), although various degrees of malignant transformation were observed among the individual animals. A high incidence of splenomegaly (91.7 %) and hepatomegaly (56.3 %) was observed. Hemorrhages of the lungs were seen in 79.2 percent (38/48) and enlargement of lymph nodes in 62.5 percent (30/48).

Histologic Findings of Ki-MSV(Ki-MuLV) Induced Rat Brain Tumors

Brain tumors induced with Ki-MSV(Ki-MuLV) in rats were shown to be hemangiomas or hemangioendotheliomas. There were multinucleated giant cells, a palisading arrangement of the cells which formed pseudo-rosettes, necrotic areas in the center, polycystic areas, pleomorphism of the cells, and considerable mitotic activity, all suggestive of glioblastoma multiforme.

DISCUSSION

It was demonstrated that xenotropic pseudotype MSV, Ki-MSV(BV2), induced brain tumors in SD-rats as was reported in our previous work (1-3, 13). It was also shown that brain tumors were induced not only by intracerebral but also by intraperitoneal ,subcutaneous or intramuscular inoculation of this virus. In this paper, we reported restriction of the host range *in vivo* by helper leukemia virus in the xenotropic pseudotype MSV. The Ki-MSV(BV2) was prepared from Ki-NRK(NP) cells by rescuing with xenotropic helper virus, BALB : virus 2.

In general, the murine sarcoma visuses (MSV) are defective and need leukemia viruses as helpers to provide missing gene products required for replication. MSV can be rescued from MSV transformed nonproducer (NP) cells by superinfection with type-C viruses, yielding a mixture of helper viruses and pseudo-type sarcoma viruses. Pseudotype sarcoma viruses are often indicated by the abbreviation for the sarcoma virus followed by that for the helper virus in parentheses : for instance, Ki-MSV(BV2), the case in which the Kirsten sarcoma genetic material is encapsulated in the helper virus (BV2) envelope (14-16).

Mouse cells contain the genetic information of endogenous type-C RNA viruses (17-21). It was recently demonstrated that BALB/c mice had three different kinds of endogenous virus genes (6). BALB virus-1 replicates mainly in cultured cells of NIH Swiss mouse origin. It was shown that BALB virus-2 was not infective

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Fig. 1. *Ki-MSV(Ki-MuLV) induced rat brain tumor* (cerebrum, ×100, H&E stain, ID No. R071278-8). A few clusters of endothelial cells from newly formed capillaries are seen. Marked proliferation of the endothelium is characteristic of this hemangioendothelioma.

Fig. 2. *Ki-MSV(Ki-MuLV) induced rat brain tumor* (cerebellum, ×400, H&E stain, ID No. R071278-17). Vascular endothelial proliferation is present. The nuclei of the endothelial cells are enlarged.

Fig. 3. *Ki-MSV(Ki-MuLV) induced rat brain tumor* (cerebrum, ×100, H&E stain, ID No. R073178-3). This area of pseudopalisading with central necrosis is typical of glioblastoma multiforme.

Fig. 4. *Ki-MSV(Ki-MuLV) induced rat brain tumor* (cerebrum, ×400, H&E stain, ID No. R071278-2). Anaplastic pleomorphic-cell "glioblastomas" with enlarged nuclei are observed.

to mouse cells, but did replicate in cells of other animal species (xenotropism) (22, 23). BALB virus-3 is different immunologically from virus-1 and virus-2 and is similar to the endogenous type-C virus isolated from NZB or NIH Swiss mice (24).

Kirsten murine sarcoma virus (Ki-MSV) transformed NRK cells to nonproducer cell lines such as Ki-NRK. Pseudotype Ki-MSV, such as Ki-MSV(Ki-MuLV) and Ki-MSV(BV2), can be rescued from NP cells with the type-c helper viruses Kirsten MuLV and BALB virus-2, respectively.

As was described previously (1-3, 13), when xenotropic pseudotype Ki-MSV, Ki-MSV(BV2) was inoculated intracerebrally into newborn or fetal rats, brain tumors frequently developed. When newborn rats were inoculated with the same virus subcutaneously, intraperitoneally or intramuscularly, either brain tumors or myogenic sarcomas were induced depending on the route of inoculation. However, no tumors developed in the Swiss Icr mice inoculated with Ki-MSV(BV2) either intracerebrally or intramuscularly at birth.

In *in vitro* experiments (25), Ki-MSV(BV2) replicated in rat cells but not in mouse cells, which seems to agree with the results of our *in vivo* experiments. As was pointed out by Aaronson and Rowe (4), the helper leukemia virus restricts host range and neutralization. BALB : virus 2 used for rescuing was not infectious to mouse cells such as NIH/3T3 or BALB/3T3 cells but readily infectious to rat cells (22, 26), thus, the host range of pseudotype MSV appears to depend upon a helper leukemia virus, *in vivo*. Similarly, Aaronson and Weaver (6) demonstrated that the ability of Ki-MSV to transform human cells is a function of its helper virus, instead of the sarcoma virus itself.

Ecotropic Ki-MSV(Ki-MuLV) was prepared *in vitro* in order to compare its oncogenicity with xenotropic pseudotype Ki-MSV(BV2). The rescued focus-forming virus prepared from nonproducer Ki-NRK cells using Ki-MuLV, a xc-plaque-forming virus isolated from Ki-MSV, showed the same host range as the original Ki-MSV (27). Ki-MSV(Ki-MuLV) obtained in our study had a titer of $10^{3 \cdot 7}$ FFU/ml on BALB/3T3 cells and $10^{4 \cdot 5}$ FFU/ml on NRK cells. Thus, both mouse and rat cells were susceptible to the newly prepared virus (28). It was also evident that *in vivo* Ki-MSV(Ki-MuLV) induced brain tumors and myogenic sarcomas in rats and mice when inoculated intracerebrally and intramuscularly, respectively.

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